Incorporation of bioactive glass in calcium phosphate cement: Material characterization and in vitro degradation

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Abstract: Calcium phosphate cements (CPCs) have been widely used as an alternative to biological grafts due to their excellent osteoconductive properties. Although degradation has been improved by using poly(D,L-lactic-co-glycolic) acid (PLGA) microspheres as porogens, the biological performance of CPC/PLGA composites is insufficient to stimulate bone healing in large bone defects. In this context, the aim of this study was to investigate the effect of incorporating osteopromotive bioactive glass (BG; up to 50 wt %) on setting properties, in vitro degradation behavior and morphological characteristics of CPC/BG and CPC/PLGA/BG. The results revealed that the initial and final setting time of the composites increased with increasing amounts of incorporated BG. The degradation test showed a BG-dependent increasing effect on pH of CPC/BG and CPC/PLGA/BG pre-set scaffolds immersed in PBS compared to CPC and CPC/PLGA equivalents. Whereas no effects on mass loss were observed for CPC and CPC/BG pre-set scaffolds, CPC/PLGA and CPC/PLGA/BG showed apparent degradation of PLGA microspheres and faster loss of integrity for CPC/PLGA/BG pre-set scaffolds compared with CPC/PLGA equivalents. Based on the present in vitro results, it can be concluded that BG can be successfully introduced into CPC and CPC/PLGA without exceeding the setting time beyond clinically acceptable values. All injectable composites containing BG had suitable handling properties and specifically CPC/PLGA/BG showed an increased rate of mass loss. Future investigations should focus on translating these findings to in vivo applications.

Key Words: calcium phosphate cement, bioactive glass, poly (D,L-lactic-co-glycolic) acid, degradation, in vitro


INTRODUCTION

Regenerative therapies for bone defects caused by trauma, injury, or resection of tumors involve the use of autografts, allografts or synthetic bone substitutes.1,2 Autogenous bone grafting is considered the gold standard and provides osteoconductive matrices, and osteoinductive factors, and osteoconductive matrices.1,3 However, several problems are related to this procedure, including limited availability of donor tissue, donor site morbidity, risks of infection, and the necessity for additional surgery.2,4 An alternative is the use of allogenic tissues, which are easier to obtain but it involve the risk of immune rejection, transmission of infectious diseases and/or lack of osteoconductivity and osteogenicity.1–3 In order to overcome these problems, synthetic bone substitutes have been developed as a promising treatment.2,4 Engineered bone substitutes are attractive because they are off-the-shelf available, can be designed toward desired handling properties and biological performance without the aforementioned limitations.2,4

In this context, calcium phosphate cements (CPCs) have been widely explored as an alternative synthetic graft for bone substitution in various types of bone surgery.5–8 CPCs
present excellent biocompatibility, osteoconductive properties, and can be used in an injectable way. However, CPCs do not have sufficient intrinsic porosity to allow tissue ingrowth and rapid degradation. Consequently, several approaches have been explored to improve the degradation of CPCs by introducing (micro)porosity. A promising approach to reach this objective is offered by the inclusion of biodegradable polymer microspheres within the ceramic matrix, which has been demonstrated to increase degradation rates and allow tissue ingrowth into the CPCs. Although different types of natural and synthetic polymers can be used for the production of microspheres, the use of poly(D,L-lactic-co-glycolic) acid (PLGA) has recently revealed to be of most interest based on its biocompatibility, nontoxicity, bioresorbable nature and additional effects of acidic degradation products on the CPC ceramic matrix. When PLGA microspheres are incorporated into CPC, rapid degradation of the polymer makes the microspheres lose their integrity, leaving porosity within the ceramic. Many reports have demonstrated that CPC/PLGA-microsphere composites are effective to increase material degradation and bone formation in different animal models, especially with the use of the dense acid-terminated PLGA microspheres.

Nonetheless, the osteoconductive properties of CPC/PLGA composites might not be sufficient to stimulate bone healing in larger bone defects, nonunion fractures and patients with compromised medical conditions (e.g., osteoporosis). In this context, many approaches have explored the potential of materials with high bioactivity rate, including bioactive glasses (BGs), either in monolithic or composite form for bone regenerative applications. BGs are a group of synthetic silica-based bioactive materials, with the unique ability to directly bond to bone tissue. Their favorable bone behavior is related to the formation of a silica gel layer, which acts as a template for calcium phosphate (CaP) precipitation, which attracts osteoprogenitor cells and osteoblasts, resulting in an increased organic matrix deposition that is subsequently mineralized. In view of the growing interest in the development of composites with improved osteogenic properties to be used as off-the-shelf available bone substitutes, it was hypothesized that the addition of BG to CPC and CPC/PLGA might offer a novel way of improving material performance. The introduction of BG was designed to integrate its high bioactivity rate to the cement, increasing the material osteogenic potential. Despite these possible advantages, there is still limited understanding of the interactions between the component phases of the composite.

Consequently, the aim of the current study was to investigate different CPC/BG and CPC/PLGA/BG formulations for their setting properties, physico-chemical, and morphological characteristics. To this end, pre-set CPC/BG and CPC/PLGA/BG composite disks were evaluated by means of XRD characterization, scanning electron microscopy (SEM), setting time measurements and in vitro degradation studies.

**MATERIALS AND METHODS**

**Materials**

Calcium phosphate cement (CPC) consisted of 85% alpha-tricalcium phosphate (α-TCP; CAM Bioceramics BV, Leiden, The Netherlands), 10% dicalcium phosphate anhydrous (DCPA; J.T. Baker Chemical, USA) and 5% hydroxyapatite (HA; Merck, Darmstadt, Germany). 2% Na₂HPO₄ was used as liquid phase for the preparation of the cement. Acid-terminated poly(D,L-lactic-co-glycolic acid) (PLGA) (Puramord®, Purac, Gorinchem, The Netherlands) with a lactic to glycolic acid ratio of 50:50 and a molecular weight (Mₙ) of 17 kDa was used for microparticle preparation. The employed BG, Biosilicate® parent glass (particle size: 2.5 μm) was provided by Vitreous Materials Laboratory (LaMaV, Department of Materials Engineering, Federal University of São Carlos, São Carlos, São Paulo, Brazil).

**Preparation of dense PLGA microspheres**

Dense PLGA microspheres were prepared by a single emulsion technique, as described previously. Briefly, 0.2 g of PLGA was dissolved in 2 mL of dichloromethane (DCM; analytical grade, Merck, Darmstadt, Germany) in a 20 mL glass tube. Two milliliters of this solution was transferred into a stirred beaker containing 100 mL of 0.3% poly vinyl alcohol (PVA) (88% hydrolyzed, MW 22000, Acros, Geel, Belgium) solution. Subsequently, 50 mL of 2% isopropanol (IPN) (Merck) solution was added. The solution was stirred for 1 h. The microspheres were allowed to settle for 1 h and the clear solution was decanted. The suspension left was centrifuged and the clear solution on top was aspirated. Finally, the microspheres were washed and collected through centrifugation at 1500 rpm for 5 min, lyophilized, and stored at −20°C until use. The size distribution of the PLGA microspheres was determined by image analysis, for which microspheres were suspended in H₂O and imaged using an optical microscope (Leica/Leitz DM RBE Microscope system, Leica Microsystems, Wetzlar, Germany). The obtained images were used to determine the size distribution of the PLGA-microspheres (n > 250) using digital image software (Leica Qwin®, Leica Microsystems).

**Preparation of pre-set composites**

Different formulations of CPC and BG, and CPC/PLGA and BG (with different wt % of CPC and BG) were evaluated. Table I gives an overview of the different formulations used. Pre-set composites were made by mixing the base materials inside a 2 mL closed tip syringe (BD Plastipak, Becton Dickinson S.A., Madrid, Spain) using a mixing apparatus (Silamats, Vivadent, Schaan, Liechtenstein). Subsequently, 2 wt % Na₂HPO₄ was added into the syringe as the liquid component and mixed for 2 min. The preparation of pre-set composites was investigated by means of X-ray diffraction (XRD), setting time measurements and in vitro degradation studies.

**TABLE I. Different Formulations of the Composites**

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPC (wt %)</th>
<th>BG (wt %)</th>
<th>PLGA (wt %)</th>
<th>2% Na₂HPO₄ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPC</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.32</td>
</tr>
<tr>
<td>CPC/BG30</td>
<td>70</td>
<td>30</td>
<td>0</td>
<td>0.33</td>
</tr>
<tr>
<td>CPC/BG40</td>
<td>60</td>
<td>40</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>CPC/BG50</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>CPC/PLGA</td>
<td>70</td>
<td>0</td>
<td>30</td>
<td>0.32</td>
</tr>
<tr>
<td>CPC/PLGA/BG30</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>0.34</td>
</tr>
<tr>
<td>CPC/PLGA/BG40</td>
<td>30</td>
<td>40</td>
<td>30</td>
<td>0.36</td>
</tr>
<tr>
<td>CPC/PLGA/BG50</td>
<td>20</td>
<td>50</td>
<td>30</td>
<td>0.38</td>
</tr>
</tbody>
</table>

CPC, calcium phosphate cement; BG, bioactive glass; PLGA, dense poly(lactic-co-glycolic acid) microspheres.
another 20 s. Directly after mixing, the composite cements were injected into Teflon molds (Ø7.8 mm × 1.8 mm). After overnight setting at room temperature, the pre-set composites were removed from the molds and analyzed using scanning electron microscopy (SEM; Jeol 6400-LINK AN 10000 at 10 kV). Before use, the pre-set composites were sterilized by γ-radiation with a minimum dose of 25 kGy (Isotron B.V., Ede, The Netherlands).

Characterization of the composites

**Material characterization.** Pre-set composites were first examined by SEM observation (Jeol 6310). The pre-set composites were mounted on aluminum stubs using carbon tape and sputter-coated with gold/palladium prior to examination. The distribution map of elements present on the surface of composites was obtained using a scanning electron microscope (Ultra-High Resolution Scanning Electron Microscope; FEI Sirion) equipped with OIM Pegasus Energy Dispersive X-ray Spectroscope (EDX, Edax).

**Porosity measurements.** The macroporosity (i.e., additional porosity after PLGA-microparticles degradation) and total porosity (i.e., intrinsic porosity/microporosity) were determined by measuring the weight of pre-set composite disks with and without PLGA-microparticles according to the method described previously. Composites were placed in a furnace at 650°C for 2 h. Subsequently, microporosity and total porosity were calculated using the following Eq. (1).

\[
el_{\text{tot}} = \left(1 - \frac{m_{\text{burnt}}}{V \cdot \rho_{\text{app}}} \right) \times 100\%
\]

\[
el_{\text{macropor}} = \left(1 - \frac{m_{\text{burnt}}}{m_{\text{nanoporous}}} \right) \times 100\%
\]

**Physicochemical characterization**

Phase identifications of pre-set composites in powder were performed by using X-ray Diffraction (Philips, Cu-Kα, 45 kV, 30 mA). PLGA samples were excluded from the analysis for reasons of interference with the ceramic matrix. X-ray diffraction (XRD) patterns were collected in the 2θ range of 20–60° and were used to evaluate the potential crystalline phases available in the composites.

**Setting time**

The initial and final setting time of the various cement formulations was assessed using custom available Gillmore needles (ASTM C266). In brief, a bronze block was used as mould containing six holes (6 mm in diameter, 12 mm in height). The mould was placed in a water bath at body temperature (37°C). Samples (n = 3) of each formulation were mixed and injected into the mould in a retrograde fashion, after which the initial and final setting time was determined.

**In vitro degradation study**

For the degradation study, pre-set composites were prepared as earlier described. The pre-set composites were placed in 3 mL of phosphate buffered saline (PBS, 10 mM, pH 7.4) and incubated at 37°C in a water bath on a shaker table (70 rpm) for 9 weeks. The assays were performed in triplicate (n = 3). After week 2, 4, 6, and 9, pre-set composites of each cement formulation were subjected to analysis.

**pH measurements.** Directly after removal of the pre-set composites from the water bath, the pH of the PBS medium was measured.

**Mass loss quantification.** The pre-set composites were vacuum dried overnight before measuring the mass. The mass loss of the pre-set composites was calculated using Eq. (3). The mass loss of the PLGA microspheres inside the samples was also determined using Eq. (4), which is a derivation of Eq. (3) under the assumption of equal cement degradation in both microporous/composite samples.

\[
R_{L} = \frac{M_{0} - M_{t}}{M_{0}} \times 100\%
\]

\[
R_{L_{\text{PLA}}} = \frac{L_{\text{comp, n}} - b \cdot M_{\text{comp, 0}} l_{\text{micro, n}}}{a \cdot M_{\text{comp, 0}} M_{\text{micro, 0}}} \times 100\%
\]

**Morphology after incubation**

The morphology of the different pre-set composites, at different set points after immersion, was determined using SEM. For this purpose, three intersections were used that were parallel to the longitudinal axis of the pre-set composites. Degradation behavior of the PLGA microspheres inside the pre-set composites was visualized at various magnifications (at least 100×).

**Statistical analysis**

Data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS, version 16.0 (SPSS Inc., Chicago, IL). Significant differences were determined using a one-way analysis of variance (ANOVA) with a Tukey multiple comparison post-test. Differences were considered significant at p-values < 0.05.

**RESULTS**

**Material characterization**

Figure 1(A) illustrates the morphological structure of the BG granulate and reveals that the BG particles have an irregular structure with an average particle size of around 2.5 μm. The preparation of PLGA-microparticles with the single-emulsion solvent-extraction technique resulted in
dense PLGA-microparticles with an average size of 40 ± 4 μm. Morphological examination using SEM revealed that the PLGA-microparticles had a spherical appearance with smooth surface [Fig. 1(B)]. SEM evaluation of the CPC showed a nanoporous structure [Fig. 1(C)]. Energy dispersive spectroscopy clearly showed the presence of Si (silicon), Ca (calcium) and P (phosphorus) with particle-specific localization of Si (from the BG-particles) and a homogenous distribution of Ca and P (from the CPC; Fig. 2). Taken together, surface examination of CPC/BG, CPC/PLGA and CPC/

FIGURE 1. Microscopic SEM micrographs of (A) BG granulate, (B) dense PLGA-microparticles, (C) CPC, (D) CPC/BG30, (E) CPC/PLGA, and (F) CPC/PLGA/BG30. BG (arrow) and PLGA-microparticles (arrowheads) are indicated in the SEM micrographs. Bar represents 1, 10, or 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2. Distribution of elements in a random location of the BG-containing composite. Silicon (Si) represents BG while the calcium (Ca) and phosphorous (P) rich areas correspond to CPC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
PLGA/BG showed homogenous distribution of PLGA-micro-particles and/or BG granulate within CPC [Figs. 1(D–F) and 2, respectively].

Porosity
Total porosity of the CPC and CPC/BG samples was similar ($p > 0.05$) with values of 41.2 ± 0.8%, 43.2 ± 1.3%, 42.7 ± 0.9%, and 42.8 ± 1.7% for CPC, CPC/BG30, CPC/BG40, and CPC/BG50, respectively (Table II). Similarly, no differences were found between the different formulations of CPC/PLGA and CPC/PLGA/BG samples for microporosity and total porosity measurements ($p > 0.05$; Table II) with respective values of 40.5 ± 1.5 and 55.5 ± 0.9% for CPC/PLGA, 41.2 ± 1.8% and 56.5 ± 1.2% for CPC/PLGA/BG30, 40.0 ± 1.4% and 55.5 ± 1.5% for CPC/PLGA/BG40 and 42.0 ± 1.8 and 57 ± 1.3 for CPC/PLGA/BG50 (Table II).

XRD
XRD patterns of the CPC confirmed the presence of all three phases, that is $\alpha$-TCP, DCPA, and HA, represented by the characteristic peaks as shown in Figure 3. BG appeared to be crystalline showing sharp diffraction peaks at 23.8, 26.8, 33.6, 34.2, and 48.7°. Spectra of samples from the three composites contained characteristic peaks of both CPC and BG (Fig. 3). For CPC/BG composites, the ratio between the intensity of BG peaks to those of CPC increased with increasing amounts of incorporated BG.

Setting time
The setting time measurements showed that the addition of BG increased the setting time of CPC and CPC/PLGA cements. The initial setting times of the CPC and CPC/BG30 were significantly lower compared to CPC/BG50 ($p < 0.05$; Fig. 4(A)). In addition, the final setting time of CPC was significantly lower compared to all CPC/BG formulations ($p < 0.05$; Fig. 4(A)). Similar results were found for the samples containing PLGA. CPC/PLGA and CPC/PLGA/BG30 composites showed significant lower values for initial setting time compared to CPC/PLGA/BG50 ($p < 0.05$). Furthermore, the final setting time of CPC/PLGA samples was significantly lower compared with all CPC/PLGA/BG formulations ($p < 0.05$; Fig. 4(B)).

Degradation study
The results of the pH measurements during degradation are presented in Figure 5. The medium of the CPC samples

### Table II. Results of the Porosity Measurements

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Porosity (%)</th>
<th>Microporosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPC</td>
<td>41.2 ± 0.8</td>
<td>–</td>
</tr>
<tr>
<td>CPC/BG30</td>
<td>43.2 ± 1.3</td>
<td>–</td>
</tr>
<tr>
<td>CPC/BG40</td>
<td>42.7 ± 0.9</td>
<td>–</td>
</tr>
<tr>
<td>CPC/BG50</td>
<td>42.8 ± 1.7</td>
<td>–</td>
</tr>
<tr>
<td>CPC/PLGA</td>
<td>55.5 ± 0.9</td>
<td>40.5 ± 1.5</td>
</tr>
<tr>
<td>CPC/PLGA/BG30</td>
<td>56.5 ± 1.2</td>
<td>41.2 ± 1.8</td>
</tr>
<tr>
<td>CPC/PLGA/BG40</td>
<td>55.5 ± 1.5</td>
<td>40 ± 1.4</td>
</tr>
<tr>
<td>CPC/PLGA/BG50</td>
<td>57 ± 1.3</td>
<td>42 ± 1.8</td>
</tr>
</tbody>
</table>

CPC, calcium phosphate cement; BG, bioactive glass; PLGA, dense poly(lactic-co-glycolic acid) microspheres.
showed a substantial pH decrease during the weeks, reaching a value of 4.5 after 9 weeks of immersion [Fig. 5(A)]. In contrast, the pH of the CPC/BG composites, at 30, 40, and 50 wt % of BG, showed to increase during immersion with significantly higher values compared with CPC samples at all time points evaluated [p < 0.05; Fig. 5(A)]. For the CPC/PLGA samples, a substantial pH decrease was observed during immersion, irrespective of BG incorporation. The pH for CPC/PLGA demonstrated to decrease faster compared to all CPC/PLGA/BG formulations (p < 0.05) within 4 weeks of immersion [p < 0.05; Fig. 5(B)]. After longer immersion (i.e., 6–9 weeks), significant differences were only observed between CPC/PLGA and CPC/PLGA/BG50 [p < 0.05; Fig. 5(B)].

Mass loss evaluation showed similar results for the CPC and all CPCP/BG formulations with ~10% loss after 9 weeks of immersion [p > 0.05; Fig. 6(A)]. Substantially faster and more mass loss (upto ~40–50% after 9 weeks) was observed for CPC/PLGA and all CPC/PLGA/BG formulations [Fig. 6(B)]. All CPC/PLGA/BG formulations showed a significantly faster mass loss at weeks 2 and 4 compared to CPC/PLGA (p < 0.05). At week 9, only CPC/PLGA/BG with 40 and 50 wt % BG showed significant decrease in mass loss as compared to CPC/PLGA [p < 0.05; Fig. 6(B)].

**Morphology**

SEM micrographs showed no apparent morphological changes in the CPC and CPC/BG samples during immersion (data not shown). On the other hand, samples containing PLGA microspheres showed apparent morphological changes during immersion. As similar morphological changes were observed for all CPC/PLGA/BG formulations, Figure 7 represents SEM images of only CPC/PLGA and CPC/PLGA/BG30.

Two weeks after incubation, PLGA microspheres in the CPC/PLGA pre-set composites showed signs of initial degradation. The same behavior was observed for all CPC/PLGA/BG formulations [Fig. 7(A,B)]. A pronounced PLGA degradation was observed after 4 weeks of immersion for all groups, resulting in the formation of spherical pores [Fig. 7(C,D)]. After 6 weeks of incubation, SEM micrographs revealed that the microspheres were completely eroded in all CPC/PLGA and CPC/PLGA/BG formulations, creating an interconnected porous structure [Fig. 7(E,F)]. After week 9, the presence of PLGA microspheres was not noticed in any of the CPC/PLGA and CPC/PLGA/BG formulations [Fig. 7(G,H)]. In addition, CPC/PLGA/BG formulations showed higher degradation compared to CPC/PLGA as shown by loss of integrity of the pre-set composites [Fig. 7(H)].

**DISCUSSION**

The present study evaluated the effect of BG incorporation into CPC and CPC/PLGA via evaluation of setting time characteristics, physico-chemical properties, degradation behavior, and morphology of pre-set composites. The results demonstrated that BG (at a maximum of 50 wt %) can be incorporated into CPCs and CPC/PLGA cements as confirmed by XRD and SEM, with clinically acceptable effects on initial setting time (<10 min). Furthermore, the
incorporation of different amounts of BG had a significant effect on chemical properties and morphological structure of the material, increasing pH and accelerating mass loss upon immersion in physiological solutions. The results clearly indicate that the characteristics presented by composites containing BG had a strong influence on the acceleration of the material degradation rate and on the creation of macroporosity, especially in the CPC/PLGA/BG samples.

The characterization of the setting properties of the composites is of interest in view of the control over the hardening of the cement.32–34 For injectable materials, a short setting time is required so that the wound closure is not delayed too much.32 Driessens et al.35 suggested that under the operation procedures, the setting time should be within a limited time range of 3 and 8 min for initial setting time and lower than 15 min for final setting time. It was
demonstrated that the formulations containing 30 wt % of BG presented the lower values for initial setting time when compared to those with higher BG amounts (6 and 7 min for CPC/BG30 and CPC/PLGA/BG30, respectively). In addition, all formulations containing BG showed a higher final setting time compared to CPC and CPC/PLGA. Setting time depends on the liquid phase, liquid/powder ratio and powder or composite composition. It is well known that the introduction of PLGA microspheres into cements results in an increased setting time compared to CPC cements, mainly due to higher amount of liquid hardener required to maintain injectable properties. For the formulations containing BG, also a marginally increased liquid phase was introduced into the syringe, which decreased the viscosity and increased the fluidity of the formulations. Consequently, the higher amount of liquid hardener resulted in a significantly higher final setting time for the different formulations.

The pH measurements confirmed that incorporation of BG into CPC resulted in alkalization of the immersion medium, whereas plain CPC induces acidification. For CPC/PLGA, the introduction of BG lowered the acidification process. The reactions taking place at the composite interface are likely responsible for these observations. Release of cations (Si, Na, Ca and P) occurs immediately after the contact of BG with fluids, resulting in an increased pH. CPC/PLGA/BG formulations, at 30 and 40 wt%, showed a linear decrease in pH, starting from week 2 and stabilizing at week 6 (pH of 5), resulting in a more physiological pH when compared to CPC/PLGA samples. It is well documented that PLGA degradation results in an acidic environment caused by the release of monomers lactic and glycolic acids. For this reason, PLGA is often combined with other materials that can counteract excessive acidification and, in turn, reduce the potential adverse tissue response. By combining CPC/PLGA with BG, the acidic and basic degradation products apparently counteracted each other resulting in a more homeostatic environment.

Mass loss evaluation showed that no significant mass loss was observed for CPC and CPC/BG formulations during the experimental periods. Conversely, CPC/PLGA/BG formulations presented a significant decrease in mass compared to CPC/PLGA, starting from week 2. At this time point, SEM micrographs revealed an initial erosion of the PLGA in the samples with BG, which continued up to 9 weeks. In these formulations, the PLGA degradation was followed by an increase in porosity. The CPC/PLGA formulations showed an initial PLGA degradation starting from week 4 onwards. The observed disparity in these findings might be related to the degradation of the polymeric phase associated with the rate of dissolution of the BG over time. The hydrolysis of PLGA creates an acid environment which accelerates CPC dissolution. Also, it is known that a rapid ion release is initiated immediately after the contact of BG with fluids, starting the degradation of the material. The degradation of PLGA may alter the release of Si, P, Na, and Ca ions from the BG phase and in turn affect the dissolution of BG. Thus, the factors that affect dissolution properties of CaP ceramics under acidic conditions might be similar to those affecting degradation of the BG.

The SEM analysis showed that the CPC/PLGA/BG samples presented a more interconnected porous structure compared with CPC/PLGA. Furthermore, at week 2, pores were already observed in the samples with BG, progressing to a loss of the structure after 9 weeks of immersion. A porous structure is important to support tissue integration and to allow vascularisation. Also, interconnecting and appropriate pore distribution is necessary for cells to be able to proliferate, differentiate and migrate into all areas of the scaffold. Porous biomaterials are also desirable for the efficient diffusion of nutrients, gases, growth factors, and other essential components to all cells within the scaffold, as well as diffusion of metabolic waste products away from the cells. In this context, the higher porosity after immersion demonstrated by the CPC/PLGA/BG samples might constitute a more appropriated structure for bone regeneration. The observed effects of BG incorporation on CPC/PLGA degradation justify further research using preclinical models to study the biological performance of these materials.

CONCLUSIONS

On the basis of the present in vitro results, it can be concluded that BG can successfully be introduced into CPC and CPC/PLGA, with clinically acceptable effects on setting time and appropriate physicochemical characteristics of the formulations, especially for 30 wt % BG incorporation. BG incorporation, especially in CPC/PLGA composites, accelerated the rate of material degradation, rapidly transforming upon immersion in physiological solutions toward a scaffold with interconnected pores and macroporosity, which would constitute a material that allows faster tissue ingrowth and the complete replacement by newly formed bone. Future research should focus on transferring and evaluating these findings to the in vivo situation.

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